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Detection of Epstein-Barr virus DNA in cardiac and aortic tissues from chronic, active Epstein-Barr virus infection associated with Kawasaki disease–like coronary artery aneurysms

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We describe three patients with chronic, active Epstein-Barr virus infection associated with Kawasaki disease–like coronary artery aneurysms. The Epstein-Barr virus genome was detected in three cardiac tissue samples and one aortic tissue sample examined by means of the polymerase chain reaction. These findings suggest that chronic Epstein-Barr virus infection may play a pathogenic role in the development of coronary artery aneurysms. (J PEDIATR 1993;123:90-2)

Chronic, active Epstein-Barr virus infection is characterized by fever, lymphadenopathy, splenomegaly, hepatitis, interstitial pneumonitis, and uveitis. Laboratory findings include anemia, thrombocytopenia, leukopenia, and hypergammaglobulinemia with a very high titer of EBV antibodies[1,2]; cardiac involvement is rare. To our knowledge, our previous report of CEBV associated with coronary artery aneurysms was the first.[3] We now report three patients with CEBV associated with coronary artery aneurysms, similar to the findings of Kawasaki disease.[4]

METHODS

Subjects. Three Japanese patients with CEBV and coronary artery aneurysms were studied (Table). Coronary artery aneurysms were detected by cross-section echocardiography. All patients had a chronic illness characterized by prolonged fever, coronary artery aneurysms, hepatosplenomegaly, liver dysfunction, and hypergammaglobulinemia. In addition to coronary artery aneurysms, patients 1 and 3 had dilation of the sinus of Valsalva, and patient 1 died of rupture of the sinus of Valsalva. Patients 2 and 3 also had pericarditis. These patients had several other manifestations of EBV infection: interstitial pneumonitis in patient 1, interstitial nephritis[5] in patient 2, and rash in patient 3 (Table). None had mucous membrane involvement.The DNA was extracted from different sites in three patients with CEBV and two control cardiac tissues. Human DNA samples from Raji cells and Molt-4 cells were used as positive and negative control samples, respectively. Human embryonic fibroblast cells infected with herpes simplex virus type 1 (Seibert strain), varicella-zoster virus (wild type), and human cytomegalovirus (wild type) and human herpes virus-6-infected cord blood mononuclear cells were used for these studies.

CEBV	Chronic, active Epstein-Barr virus infection
EBV	Epstein-Barr virus
KD	Kawasaki disease
PCR	Polymerase chain reaction

EBV serologic tests. Antibody titers to the EBV viral capsid antigen and early antigen were determined by indirect immunofluorescence. Antibody titers to EBV-determined nuclear antigen were determined by anticomplement immunofluorescence.[3]

Polymerase chain reaction. The PCR was performed according to a previously described method.[6] The PCR reaction mixture consisted of 200 μmol of each deoxyribonucleotide, 2.5 U of *Taq* DNA polymerase, 50 nmol of potassium chloride per liter, 10 mmol of Tris-HCl (pH 8.3) per liter, 1.5 mmol of magnesium chloride per liter, 0.01% (wt/vol) of gelatin, 20 pmol of each oligonucleotide primer, and 1 μg of DNA in a volume of 100 μl. Samples were then subjected

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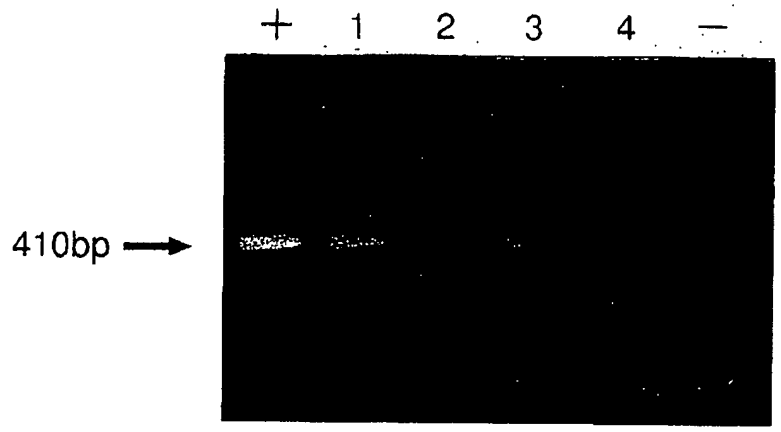

Figure. Ethidium bromide staining of PCR products. Aliquots (10 μl) of PCR products were subjected to electrophoresis. *Lane 1*, Aortic tissue DNA from patient 2; *lane 2*, cardiac tissue DNA from patient 2; *lane 3*, cardiac tissue DNA from patient 3; *lane 4*, cardiac tissue DNA from patient 1; +, Raji cell DNA, positive control sample; -, Molt-4 cell DNA, negative control sample; *bp*, base pairs.

to 35 cycles of PCR, each consisting of 1 minute of denaturation at 94° C, 2 minutes of annealing at 55° C, and 3 minutes of polymerization at 72° C by a DNA thermal cycler (Perkin-Elmer Cetus, East Norwalk, Conn.). The PCR procedure was performed with primers 5′ -GTGACTTCACCAAAGGTCAG-3′ and 5′-TTAAAGTCCACTTACCTCTG-3′. The primers amplify and detect a 410–base-pair sequence in the region within the EBV *Bam*HI-W fragment.

Southern blot hybridization. Electrophoresis of 10 μl of the PCR product was performed on 1.8% agarose gel. The gel was stained with 1 μg of ethidium bromide per milliliter for 5 minutes, and the DNA bands were visualized under ultraviolet light. The PCR product was transferred onto nitrocellulose membrane filters. Each filter was hybridized with ^{32}P-labeled cloned fragment *Bam*HI-W of the EBV genome for 48 hours at 41° C in 1× standard saline citrate (0.15 mol sodium chloride per liter and 0.015 mol sodium citrate per liter), 50% formamide, 0.5% sodium dodecylsulfate, and heat-denatured salmon sperm DNA, 100 μg/ml. After hybridization, the filters were washed three times at room temperature in 0.1× standard saline citrate with 0.1% sodium dodecylsulfate and then incubated three times for 1 hour at 50° C. The filters were dried and exposed to x-ray film at −80° C.[7]

RESULTS

In all these patients, EBV serologic tests revealed extremely high IgG antibody titers against early antigen and viral capsid antigen (Table). There were no significant elevations in antibody titers to other herpesviruses or to measles virus in these patients, or to adenovirus in patients 1 and 2.

Table. Clinical and laboratory findings in three patients with CEBV

	Patient No.		
	1	2	3
Age (yr)/gender	2/M	6/F	5/F
Prolonged fever	+	+	+
Coronary artery aneurysms	+	+	+
Dilation of sinus of Valsalva	+	−	+
Pericarditis	−	+	+
Rash	−	−	+
Mucous membrane involvement	−	−	−
Desquamation of fingertips	−	−	−
Lymphadenopathy	+	+	−
Hepatosplenomegaly	+	+	+
Liver dysfunction	+	+	+
Interstitial nephritis	−	+	−
Interstitial pneumonitis	+	−	−
Leukopenia	−	−	−
Anemia	+	−	+
Thrombocytopenia	−	−	−
Thrombocytosis	−	−	−
Hypergammaglobulinemia	+	+	+
EBV antibody titer			
VCA-IgG	10,240	20,480	5,120
EA-IgG	2,560	5,120	5,120
EBNA	160	40	10

VCA, Viral capsid antigen; *EA*, early antigen; *EBNA*, EBV-determined nuclear antigen.

The PCR-amplified genomic Raji cell DNA was confirmed on ethidium bromide–stained gel and by Southern blot hybridization with a radiolabeled EBV *Bam*HI-W fragment. When DNA samples from cells infected with herpes simplex virus type 1, varicella-zoster virus, human cytomegalovirus, and human herpesvirus-6 were used as

templates in the PCR reaction, no amplification was noted by direct gel analysis or Southern blot hybridization.[6] The EBV genome, as indicated by the 410–base-pair DNA band identical to that of the positive control sample, was detected in cardiac tissue DNA from all three patients and in aortic tissue DNA from one patient (No. 2) on direct gel analysis (Figure). In two control cardiac tissue samples, the PCR procedure did not detect the EBV genome. In tissue samples from the spleen, kidney, and lung of these three patients, the PCR procedure detected the EBV genome. The EBV genome was also detected in aortic tissue of patient 2[7] and in cardiac tissue of patient 3 by Southern blot hybridization without prior DNA amplification.

DISCUSSION

Vascular and endovascular complications are rare in EBV infection. Loeffel et al.[8] reported an 8-year-old boy with X-linked lymphoproliferative syndrome and aneurysms of central nervous system arteries; the autopsy revealed necrotizing vasculitis in the central nervous system and microscopic changes in the coronary arteries. Ilowite et al.[9] described a 12-year-old boy with Wiskott-Aldrich syndrome who subsequently had pulmonary vasculitis associated with EBV-induced lymphoreticular proliferation; the lung biopsy specimen showed vasculitis involving small to medium-sized arteries and veins.

We now have identified three patients with CEBV accompanied by coronary artery aneurysms. All three had high antibody titers to replicative antigens of EBV and no significant elevations in antibody titers to other herpesviruses, measles virus, or adenovirus. We were not able to test for the presence of the EBV genome in coronary arteries. However, detection of the EBV genome in the heart and aorta suggests that EBV may play a pathogenic role in the development of coronary artery aneurysms in CEBV.

Kawasaki disease is thought to be a self-limited, immunologically mediated vasculitis. We previously reported a link between KD and EBV; 49 (86%) of 57 patients with KD and 15 (68%) of 22 with recurrent KD had serologic evidence of an unusual primary EBV infection.[10, 11] The EBV genomes were identified directly by means of the PCR in peripheral blood mononuclear cell DNA samples from 21 (60%) of 35 patients within 2 weeks after the onset of KD. Furthermore, EBV genomes were also detected in all six patients who were repeatedly tested within 3 months after disease onset. In contrast, only 2 (12%) of 17 control DNA samples showed positive PCR results.[6] Epstein-Barr virus genomes were frequently detected in peripheral blood mononuclear cell DNA from KD patients, indicating that there were much higher proportions of EBV-infected cells in their peripheral blood mononuclear cells than in those of normal control samples. These virologic studies indicate that an unusual EBV-cell interaction may exist in KD.

A broad spectrum of associated features and complications has been reported with CEBV. Coronary artery aneurysms constitute one of the clinical features and appear not to be as rare in these patients as previously assumed. These three patients did not have the clinical hallmarks of KD but all had coronary artery aneurysms. Their recurrent episodes of fever during 6 months to several years can be distinguished from those of atypical KD.[12] All the patients with CEBV associated with coronary artery aneurysms died within 5 years of onset. We recommend periodic echocardiographic examinations for all patients with CEBV. Further studies are needed to explain the relationship between coronary artery aneurysms and EBV infection.

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